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MLL-MLLT10 fusion in acute monoblastic leukemia: variant complex rearrangements and 11q proximal breakpoint heterogeneity

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Abstract

Cytogenetic studies of acute monoblastic leukemia cases presenting *MLL-MLLT10* (alias *MLL-AF10*) fusion show a broad heterogeneity of chromosomal breakpoints. We present two new pediatric cases (French–American–British type M5) with *MLL-MLLT10* fusion, which we studied with fluorescence in situ hybridization. In both we detected a paracentric inversion of the 11q region that translocated onto chromosome 10p12; one case displayed a variant complex pattern. We review the cytogenetic molecular data concerning the proximal inversion breakpoint of 11q and confirm its heterogeneity. © 2004 Elsevier Inc. All rights reserved.

1. Introduction

Chromosome rearrangements involving the mixed lineage leukemia (*MLL*) gene at the 11q23 locus occur in ~5–10% of acute leukemias, mainly of myeloid or poorly differentiated lymphoid origin, and are associated with poor prognosis [1–3]. To date, 37 partners of *MLL* have been identified [4]. In particular, two genes may be involved in t(10;11): *SSH3BP1* (alias *ABI-1*; also *ABII*), which is a human homolog to the mouse Abl interactor 1 at 10p11.2, and which has been described in only three cases of pediatric acute monocytic leukemia [5–7], and *MLLT10* (alias *AF10*) at 10p13, which is more commonly fused to *MLL* [8]. The majority of the other *MLL* rearrangements result from a reciprocal translocation, but *MLL-MLLT10* fusions require more complex chromosome rearrangements. These various mechanisms have recently been elucidated by Van Limbergen et al. [3]. All the 11q23 translocations involving *MLL* lead to fusion genes in which the 5' part belongs to *MLL* [9]. The expression of a chimeric product requires the same orientation of the two fused genes: because the direction of *MLL* transcription is centromere to telomere, an inversion has to occur if the partner gene is oriented in the opposite way [9]. Four recom-

bination patterns have been proposed: types 1 and 2 result from inversion of 11q13~q14-q23 involving the *MLL* locus, followed by translocation with (type 1) or insertion into (type 2) 10p12; the two other types require the inversion of *MLLT10* gene followed by translocation with (type 3) or insertion into (type 4) 11q23 [3]. We present two new cases of pediatric acute monoblastic leukemia with *MLL-MLLT10* fusion of type 2 (according to Van Limbergen et al. [3]), one of them showing involvement of a third chromosome. We also briefly discuss the variability of the breakpoint on 11q centromeric to *MLL* gene in this particular mechanism.

2. Case reports

2.1. Patient 1

At 4 months of age, hyperleukocytosis (a white blood cell [WBC] count of $41 \times 10^9/L$) prompted a diagnosis of acute myeloid leukemia (AML), French–American–British (FAB) type M5a. Blast immunophenotype was characterized by a positivity of HLA-DR, CD45, CD34, CD33, CD11a, CD11b, CD11c, CD15, CD7, CD19, and CD38. The child was enrolled in protocol AML-BFM 93 [10] and achieved clinical remission. At 4 months from diagnosis, however, leukemia recurred, with the presence of blasts in cerebrospinal fluid and partial (9%) marrow involvement. Bone marrow transplantation (BMT) from a partially matched related donor was performed after conditioning

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with busulfan–cyclophosphamide–melphalan and intrathecal cytarabine. At 5 months after BMT, a second hematological and central nervous system (CNS) relapse occurred; further remission with full donor chimerism was achieved following enrollment in IDA FLAG/FLAG protocols [11], as confirmed by the absence of recipient-derived DNA through short tandem repeat polymorphism analysis of BM cells. A second BMT from the same donor was successfully performed, and at writing the patient was alive and in third complete remission, 24 months after retransplant.

2.2. Patient 2

When the patient was 8 months old, a diagnosis was made of AML FAB M5 with CNS and cutaneous involvement. The patient was enrolled in an ongoing international protocol for infant leukemia (Interfant 99: dexamethasone, vincristine, daunorubicin, cytarabine, L-asparaginase, methotrexate, cyclophosphamide, and mercaptopurine) and achieved hematological, liquoral, and cutaneous remission. After 8 months of treatment, hematological and CNS relapse occurred. A second-line chemotherapy course (FLAD: fludarabine, cytarabine, and liposomal daunorubicin), delayed due

to interstitial pneumonia, produced a partial response with persistence of residual blasts in bone marrow, CNS and cutis (leukemoids). The child underwent allogeneic BMT from a match-unrelated donor (female) after conditioning with busulfan–thiotepa–etoposide and intrathecal cytarabine; however, lymph node relapse occurred 2 months after transplant and the child died shortly after with progressive disease.

3. Materials and methods

At onset or relapse (or both), chromosome analyses were performed on unstimulated peripheral blood (PB) and bone marrow (BM) from both patients, and on lymph node blasts from patient 2, using a QFQ-banding technique. A minimum of 20 metaphases were screened. Karyotype designation was according to International System for Human Cytogenetic Nomenclature (ISCN 1995) [12]. Fluorescence in situ hybridization (FISH) analyses were performed according to the manufacturer’s protocols using whole-chromosome 7, 10, and 11 painting probes (wcp) (Appligene Oncor-Qbiogene, Illkirch, France) and using the LSI MLL dual-color probe

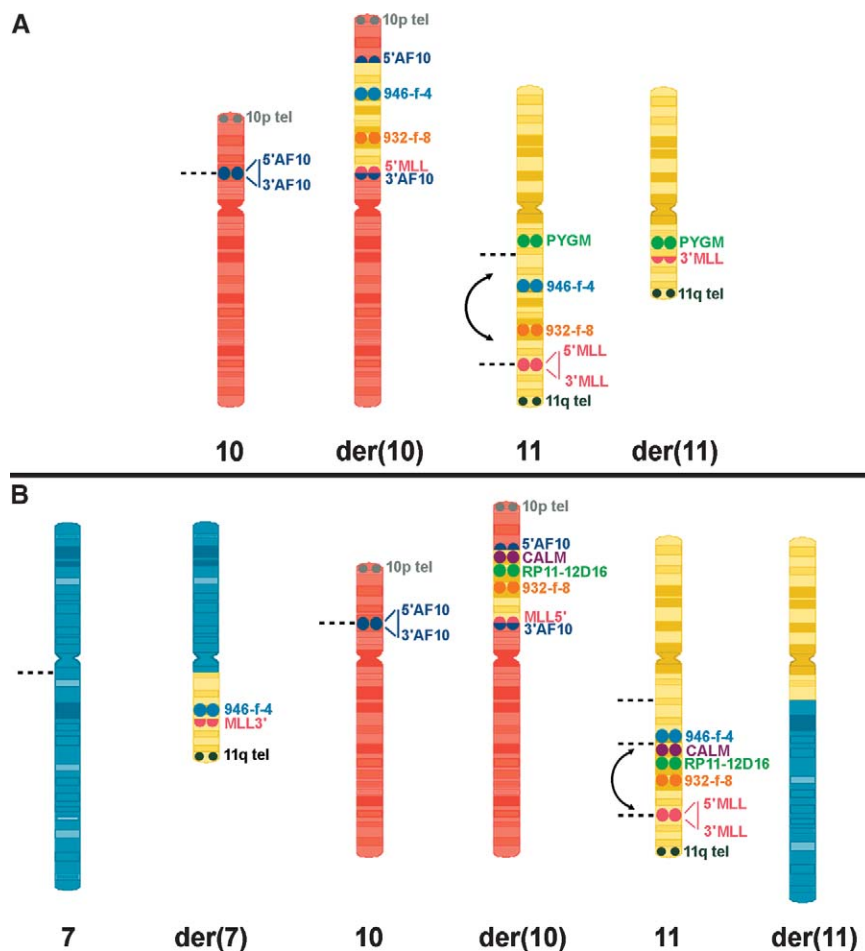


Fig. 1. Ideograms of the 11q arm, with schematic representation of FISH studies in (A) patient 1 and (B) patient 2. Dotted lines show breakpoints; arrowheads indicate the regions of 11q chromosome inversion.

(Vysis, Downers Grove, IL), which recognizes the *MLL* gene at 11q23. Human DNA inserts of yeast artificial chromosome (YAC) were amplified using Alu-polymerase chain reaction as previously described [13]. YAC or bacterial artificial chromosome (BAC) probes were labeled with nick translation with biotin-16- or digoxigenin-11-dUTP (Roche, Mannheim, Germany) and visualized using streptavidin-Cy3 (Amersham Pharmacia Biotech, Buckinghamshire, UK) and sheep antidigoxigenin-FITC (Roche). The following YAC or BAC probes were used: RP11-418C1 located telomeric to *MLLT10* and also containing the 5' part of the gene cohybridized with RP11-249M6 located centromeric to *MLLT10* and also containing the 3' part of the gene; RP11-141J21 cohybridized with RP11-665N17, both located at 11q13.1, centromeric and telomeric to the marker *PYGM* (muscle glycogen phosphorylase gene), respectively [9]; YAC 946_f_4 (11q14.1) cohybridized with YAC 932_f_8 (11q21); RP11-103I15 containing the *PICALM* gene (alias *CALM*, *CLTH*) at 11q14.2 cohybridized with RP11-12D16 located telomeric to *PICALM*; subtelomeric 10p RP11-145I2

cohybridized with subtelomeric 11q RP11-209L12. Images were captured on a Zeiss Axioplan microscope (Carl Zeiss, Jena, Germany) equipped with a charge-coupled device (CCD) camera. Adobe Photoshop software (Adobe Systems, Seattle, WA) was used to pseudocolor and merge images.

4. Results and discussion

4.1. Patient 1

Cytogenetic analysis performed on BM blasts revealed a chromosome 10;11 rearrangement. FISH studies are outlined in Fig. 1A. FISH analyses with wcp 10 and 11 and with 10p and 11q subtelomeric probes revealed insertion of chromosome 11q material into the short arm of chromosome 10. Hybridization signals for the *MLL* dual probe were split, with localization of the 5' part of *MLL* on the proximal region of 11q inserted into 10p, while the 3' part of *MLL* was retained on the derivative chromosome 11. Dual-color FISH showed that probes for the 3' (RP11-249M6) and 5'

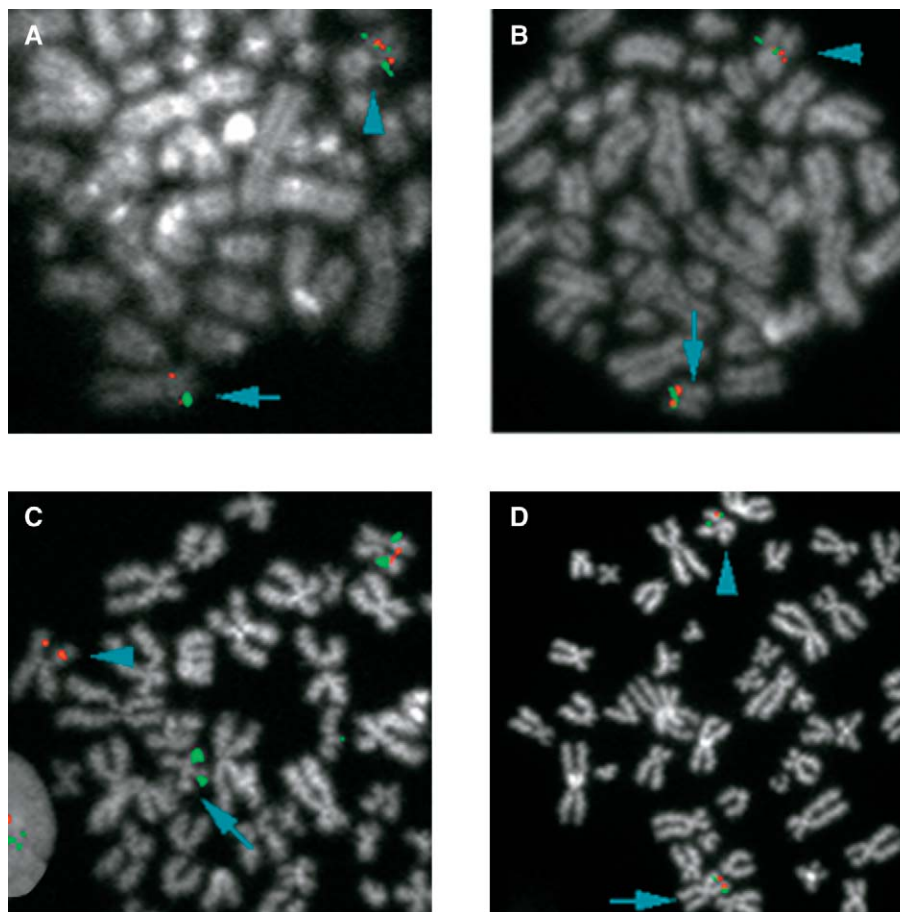


Fig. 2. FISH analyses of the 11q proximal inversion breakpoint in metaphase cells of (A, B) patient 1 and (C, D) patient 2. (A) YACs 946_f_4 at 11q14.1 (green) and 932_f_8 at 11q21 (red) were present on the normal chromosome 11 (arrowhead) and on the der(10) (arrow). (B) RP11-141J21 (red) and RP11-665N17 (green) at 11q13.1, centromeric and telomeric to the *PYGM* gene, respectively, both localized on the normal chromosome 11 (arrowhead) and on the der(11) (arrow). (C) Hybridization signals of YACs 946_f_4 at 11q14.1 (green) and 932_f_8 at 11q21 (red) are localized on the der(7) (arrow) and the der(10) (arrowhead), respectively. (D) RP11-103I15 (red) containing the *PICALM* gene at 11q14.2 and RP11-12D16 (green) telomeric to *PICALM* were present on the normal chromosome 11 (arrowhead) and on the der(10) (arrow).

Table 1
Proximal inversion breakpoints at 11q detected with FISH

Case ^a	Mechanism type ^b	Probe	Breakpoint region	Reference
a	1 variant	PYGM/3.16	11q10~11q13.1	Patient 3 in Beverloo et al., 1995 [9]
b	1	PYGM/3.16	11q13.1~11q21	Patient 5 in Beverloo et al., 1995 [9]
c	2 variant	946_f_4/932_f_8	11q14.1~11q21	Patient 1 in Van Limbergen et al., 2002 [3]
d	1	RP11-665N17/946_f_4	11q13.1~11q14.1	Patient 1, present study
e	2 variant	946_f_4/RP11-103I15	11q14.1~11q14.2	Patient 2, present study

^a Cases correspond to a–e in Fig. 3.

^b Classification according to Van Limbergen et al. [3].

(RP11-418C1) regions of *MLLT10*, which normally hybridize on the short arm of chromosome 10, were located at either side of the inserted 11q segment. Hybridization signals for YAC 946_f_4 (11q14.1) and YAC 932_f_8 (11q21) both localized on the inserted 11q segment (Fig. 2A), whereas RP11-141J21 and RP11-665N17 (PYGM) signals were retained on the derivative chromosome 11 (Fig. 2B). The karyotype thus was 46,XY,ins(10;11)(p12;q23q13).

4.2. Patient 2

The karyotype of PB and BM blasts at onset and at relapse was described as 46,XY,t(7;11;10)(q21;q21;p15). At second relapse after BMT, the karyotype of lymph node blasts revealed an additional chromosomal anomaly: 46,XY,t(1;4)(p32;q31),t(7;11;10)(q21;q21;p15). FISH analyses summarized in Fig. 1B detail the complex rearrangement that occurred. FISH with wcp 7, 10, and 11 and with subtelomeric probes for 10p-tel (RP11-145I2) and 11q-tel (RP11-209L12) revealed an insertion of chromosome 11 material into the short arm of chromosome 10 and the reciprocal translocation between the telomeric portion of 11q and part of chromosome 7 long arm. Hybridization signals for the *MLL* dual probe were split: the 5' part of *MLL* was inserted into 10p and the 3' part of *MLL* was translocated onto the derivative chromosome 7; no *MLL* signal was observed on the derivative chromosome 11. Dual-color FISH using probes for the 3' (RP11-249M6) and 5' (RP11-418C1) parts of *MLLT10* localized both signals on the short arm of the derivative chromosome 10, split at the proximal and distal ends of 11q inserted segments, respectively. Hybridization signals for YAC 946_f_4 (11q14.1) and YAC 932_f_8 (11q21) were localized on derivative chromosome 7 and on the distal part of inserted 11q segment, respectively (Fig. 2C). Cohybridization between *PICALM* probes RP11-103I15 and RP11-12D16 showed both signals retained on the distal part of the 11q inserted segment (Fig. 2D). After FISH analysis, the revised karyotype was 46,XY,t(7;10;11)(7pter→7q11.2::11q13→11q14.1::11q23→11qter;10pter→10p12::11q14.2→11q23::10p12→10qter;11pter→11q13::7q11.2→7qter).

The t(10;11)(p12;q23) is associated mainly with acute monocytic leukemias, frequently with CNS disease and/or skin involvement [14], as in the two patients described here. The breakpoint cluster region on 11q23 spans an 8.3-kb segment of genomic DNA, comprising exons 5–10 of the *MLL* gene [8]. The t(10;11) produces a *MLL-MLLT10* transcript originating from the fusion of the 5' end of *MLL* and the 3' end of *MLLT10*: the *MLL-AF10* chimeric protein contains the AF10 leucine-zipper motif [8] required for immortalization of myeloid progenitors in vitro and endowed with leukemogenic capacity in vivo [15]. Up to now, the pattern of 10;11 chromosomal rearrangements has been determined with molecular cytogenetics in 30 cases [3,16,17]. Our two cases show a type 2 pattern according to Van Limbergen's classification: the first case is a more typical ins(10;11) and the second case involves a third chromosome, requiring at least five breakpoints. A variant complex rearrangement involving more than two chromosomes, as in our patient 2, had been reported in only five previous cases [3,16,17]. The mechanism of type 1 and 2 rearrangements requires two breaks (at 11q13~q21 and 11q23, respectively), followed by paracentric inversion of the 11q segment including the 5' part of *MLL* and its insertion or translocation into 10p12. The proximal breakpoint of the

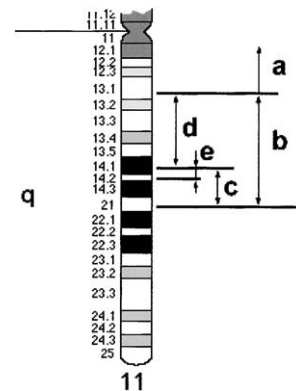


Fig. 3. Schematic illustration of proximal inversion breakpoints: *a* and *b* correspond, respectively, to patients 3 and 5 reported by Beverloo et al. [9]. *c* corresponds to patient 1 reported by Van Limbergen et al. [3]. *d* and *e* to our patients 1 and 2.

inverted segment comprising the *MLL* gene had been identified through molecular cytogenetics in only three cases [3,9] prior to the present two cases (Table 1; Fig. 3). The heterogeneity of the proximal breakpoint locations seems to be confirmed, because the regions defined do not overlap. To better define the complex rearrangements occurring between 10p and 11q, which are often unclear following standard cytogenetic analysis alone, the use of FISH techniques is needed.

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